Phone :
SLOane 4277

Lister Institute,
Chelsea Bridge Road,
London, S.W.1.

13th July, 1954.

Dear Dr. Lederberg,

Thank you for your letter of the 6th July. I am writing in a hurry, so that this letter may reach you before you leave for your holiday on July 20th.

(1) The parcel containing the collection of special strains of the typhoid-paratyphoid group of organisms will be dispatched within the next 8 or 10 days. Since the package will not contain any phages or sera, it will be sent by ordinary surface mail, together with the List giving full details of the strains. The parcel will probably arrive about mid-August and may safely be left unopened at room-temperature for several weeks, until your Department starts full work again.

(2) You may remember that in the joint paper with Craigie on typhoid phage-typing (Lancet, 1947, on page 12 of the reprint) we advised against drying of the type cultures for the time being. For reasons similar to those mentioned in my letter of the 29th June, and also for experimental purposes, I decided to have the complete sets of type strains of the internationally recognized phage types of Salm. typhi and Salm. paratyphi B also dried and kept in safe custody in several Institutes in different parts of the world. These dried cultures probably need not be tested earlier than perhaps after some 10 or 20 years. I shall send you a complete set of these cultures (in dried ampoules only) at the same time the cultures mentioned under (1) are sent.

(3) For Gram-negative organisms like Salmonella, Shigella, E. coli, Proteus etc. I advise keeping the stock collection at room-temperature in preference to storage at 2° C. to 4° C. This procedure also is followed at the N.C.T.C. at Colindale.

Since you mentioned in your letter that your collection is being maintained partly 'in nutrient agar stabs' I would like to refer you to my old paper in the J.Hyg.Camb., 1938, 38, 750. You will find on page 759 that strong emphasis is laid on the necessity of using different nutrient agar medium for the stabs in which the cultures are preserved, as contrasted with the medium employed for surface growth. It is essential to make sure that the agar-stab medium be completely free from any fermentable substances (see page 759, line 6), otherwise the cultures will not survive long enough,

or most of the surviving organisms will be found to be 'rough' variants. In this country a commercial meat extract 'Lab Lemco' has been found suitable for this special purpose. The same product would be a very poor ingredient of agar medium employed for surface growth. I enclose a copy of a brief note on Lemco agar I used to send to my correspondents years ago.

If you look up my letter dated 10th February, 1953, page 2, lines 8 to 12, you will see that the cultures that were prepared for you in January 1953 were derived from Lemco-agar stab cultures dated 1935 or 1936, that is to say, they had survived for 18 years at room-temperature.

(4) The nineteen special strains shown in the enclosed List, that are widely employed in agglutination tests, virulence tests and in the preparation of T.A.B. vaccine, have been maintained exclusively on or in agar medium throughout their whole period of laboratory existence. The ampouled dried cultures, of course, were also prepared from agar-grown cultures.

It is only since the introduction of routine phage typing of Salm.typhi and Salm.paratyphi B (about 1940) that I have been using Dorset egg medium. This has been and is being employed exclusively for the preservation of those strains that are used in routine phage typing or in experimental work with phage. The reason being that 'rough' variation occurs much more readily on, or in, agar medium than it does on Dorset egg medium. It is thus possible to employ in bacteriophage tests stock cultures from Dorset egg slants without plating and colony selection at very short intervals, whereas this would be unavoidable with agar-grown stock cultures.

(5) The history of strain 0901, which goes under the name Felix and Olitzki, is as follows: Broth cultures of H901 were stored in the dark at room-temperature and the ageing cultures were examined at regular intervals over periods of weeks and months (during 1925 in Jerusalem). This procedure had been employed successfully in earlier work on antigenic variation in Proteus X strains (see Ztschr.Immun Forsch., 1922, 35, 57, particularly page 61 and Tables I and VI).

You may be interested in that paper on Proteus X strains also for two other reasons (see Summary, pp. 95-96) :

(a) Variants of 'intermediate' antigenic constitution were described, indicating that the phenomenon was due to gradual

destruction (or suppression) of the original antigenic components and gradual development of different antigenic components.

(b) Qualitative changes in the H antigen were not observed.

(6) The reference to the earlier attempts at isolating a stable O-form of Salm.typhi is J.Immunology, 1924, 9, 115. The essential difference between 'real' O-forms and 'apparent' O-forms is discussed on pages 156-157 of that paper.

(7) The re-examination at regular intervals of an ageing broth culture is, of course, an extremely simple experimental manipulation. I would certainly recommend it as the most promising general method of isolating stable O-forms in Gram-negative organisms.

When broth cultures stoppered with cotton-wool plugs are kept, in tubes or in larger vessels, for weeks or months at room-temperature, the volume gradually shrinks and the concentration of the various components of the medium gradually increases. This probably is the factor stimulating variation.

The examination of the ageing cultures may be continued until the volume of the liquid has been reduced to 10% or 5% of its original size. I used to continue with the examination until the contents of the tubes had reached a semi-solid state.

I do not think it advisable to accelerate the process by keeping the cultures at 37° C. instead of at room-temperature. On the other hand, in experiments with the different Gram-negative organisms it may be useful to employ a number of different liquid media in addition to the customary broth. The eve of the summer vacation may be just the right time to set up such an experiment and thus gain a month or two.

(8) With regard to the E. coli strains N.C.T.C. Nos 122 and 123 I am afraid the only person who could give details of their antecedents is Dr. Cowan at the N.C.T.C. When the N.C.T.C. was transferred from the Lister Institute to the P.H.L.S. at Colindale none of the records were left at the Lister Institute. I am writing to Dr. Cowan asking him to do his best and to try to trace for you the history of these cultures.

Please do not bother to answer this letter while you are busy preparing for your holiday.

With best wishes and kind regards,

Yours very sincerely,

A. Frei

Professor Joshua Lederberg,
Department of Genetics,
University of Wisconsin,
Madison 6, Wisconsin.